14:05:19 OCA PAD INITIATION - PROJECT HEADER INFORMATION 05/11/89

Active

Project #: E-25-M80 Cost share #: Rev #: 0
Center # : R6595-1A0 Center shr #: OCA file #:
Work type : RES
Contract#: LTR DTD 880725 Mod #: Document : OTH
Prime #: 5 R01 HL41175-02 Contract entity: GTRC

Subprojects ? : N
Main project #:

Project unit: ME Unit code: 02.010.126
Project director(s):
NEREM R M ME (404)894-2768

Sponsor/division names: UNIVERSITY OF TEXAS / SAN ANTONIO, TX
Sponsor/division codes: 400 / 045

Award period: 890501 to 900430 (performance) 900430 (reports)

Sponsor amount	New this change	Total to date
Contract value	87,275.00	87,275.00
Funded	87,275.00	87,275.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: VASCULAR HEALING: CELL BIOLOGY AND RHEOLOGIC FACTORS

PROJECT ADMINISTRATION DATA

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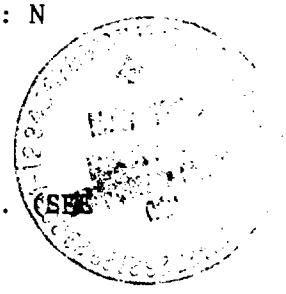
Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A supplemental sheet
Equipment title vests with: Sponsor GIT
NONE PROPOSED.

Administrative comments -
2ND YEAR FUNDING OF CONSORTIUM AGREEMENT. NIH GRANT GUIDELINES GOVERN. (SEE E-25-614 FOR DETAILS.)



GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 02/21/91

Project No. E-25-M80 Center No. R6595-1A0

Project Director NEREM R M School/Lab MECH ENGR

Sponsor UNIVERSITY OF TEXAS/SAN ANTONIO, TX

Contract/Grant No. LTR DTD 880725 Contract Entity GTRC

Prime Contract No. 5 R01 HL41175-02

Title VASCULAR HEALING: CELL BIOLOGY AND RHEOLOGIC FACTORS

Effective Completion Date 900430 (Performance) 900430 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	900725
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments CONTINUED BY E-25-M44

Subproject Under Main Project No.

Continues Project No. E-25-614

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER 1 R01 HL41175-03	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Schwartz, Colin J.	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION The University of Texas Health Science Center	4/1/90	3/31/91

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Vascular Healing--Cell Biology and Rheologic Factors

(SEE INSTRUCTIONS)

1. Brief Summary of Plans:

Based on encouraging and significant progress this year (-02), no major changes in our overall research plan are anticipated. Due to insights derived from recent developments in this laboratory and others, some modifications of research strategies within our four Specific Aims are planned to utilize and build upon this new information. Within Specific Aim #1 most proposed studies are now complete and are summarized below. Remaining to be completed within this objective are experiments examining the influence of endothelial cell growth factor (ECGF) on the proliferation of endothelial cells (EC) residing on the porous polyester substrate during exposure to different levels of shear stress. These studies are designed to examine whether the decrease in EC proliferation rate observed previously in response to elevated shear can be partially or totally reversed by ECGF. Studies within Specific Aim #2 to examine the influence of shear stress on monolayer integrity will continue as proposed. The only alteration from the initial proposal anticipated is an expansion of the number of cell adhesion molecules that will be investigated as a result of the tremendous progress that is occurring within this area of cell biology. Our initial progress indicating that elevated shear stress can significantly modify both platelet and monocyte recruitment and adherence to the endothelial surface as well as recent developments related to possible mediators involved in these interactions have allowed us to better focus our planned efforts within Specific Aim #3. Specifically, the availability of a monoclonal antibody and the recent cloning of the gene for the monocyte chemoattractant, SMC-CF, will allow us to probe more precisely the kinetics of synthesis and release of this molecule as a possible key mediator involved in the shear stress responses. Further, recent developments related to adhesion molecules involved in monocyte-endothelial cell interaction allow us to use monoclonal antibodies to examine the role and expression of these important molecules in relation to shear stress modulation. Plans for Specific Aim #4 remain unchanged including plans to initiate studies early in year -03. As proposed and performed to the present, these plans involve a coordinated and essential cooperation between investigators at UTHSCSA and GIT.

2. Current Studies:

Specific Aim #1:

1) During this year, endothelial seeding and culture conditions have been optimized to generate confluent cultures on 1μ pore polyester mesh within 3 days of seeding. Scanning electronmicroscopy has established that when such endothelized polyester graft material is subjected to high fluid mechanical shear stress (30 dynes/cm^2 - 24 hr), the endothelial cells (EC) elongate and orient in the direction of flow. These changes in endothelial cell geometry and orientation are similar to those seen with EC cultured on plastic substrates, and for arterial cells *in vivo*. Specifically, our studies indicate that optimal seeding and time required to attain a confluent endothelial cell culture on the porous polyester mesh is obtained using a single collagen type I coat. Use of fibrinonectin or Cell-Tak either alone or along with collagen afforded no advantage and actually reduced the cell density attained.

Specific Aim #2:

The ability of endothelial cells to remain attached to prosthetic vascular graft surfaces, when exposed to high hemodynamic shear stress, is of great importance for graft survival and patency. Studies performed at GIT have, therefore, been initiated to determine the level of shear stress required to detach endothelial cells from the polyester mesh graft surface. This is termed the "critical denudative shear stress." For EC cultured on polystyrene plastic the critical denudative shear stress is some 25-30 dynes/cm^2, and on non-porous polyester film it is some 160-200 dynes/cm^2. But on 1μ porous polyester mesh the EC monolayers withstand a shear stress of 300 dynes/cm^2 without any detectable cell loss. This far exceeds the shear stress levels likely to be achieved *in vivo*.

Specific Aim #3:

Platelet-endothelial and monocyte-endothelial cell interactions are pivotal in the processes of thombosis and atherogenesis. Platelet and monocyte adhesion studies performed on endothelialized polyester vascular graft material preconditioned to either high (30 dynes/cm^2) or low (< 1 dyne/cm^2) shear stress for 24 hr, indicate that both platelet and monocyte adherence to high shear conditioned grafts was reduced by 29.4 and 25.6% respectively, relative to low shear conditioned grafts. These findings have

important implications in terms of graft survival and patency. Further, recent investigations reveal that expression of the cellular mechanism(s) responsible for the decreased adherence of both platelets and monocytes to EC exposed to elevated shear stress appears to be time dependent. Thus, recent data indicate exposure of EC to 2 h or less to elevated shear stress (30 dynes/cm^2) prior to adhesion assays induces no change in monocyte nor platelet adherence relative to cells exposed to low shear (< 1 dyne/cm^2) stress. In contrast EC exposed for 4 h to an elevated shear stress do exhibit a significant reduction in both platelet and monocyte adherence relative to low shear controls. This information should prove extremely valuable in focusing our use of molecular probes to reveal the cellular mechanisms involved in mediating these biologic responses to shear stress.

Specific Aim #4:

Finally, critical to the potential use of the polyester mesh as a key component of a hemodynamically preconditioned endothelialized prosthetic graft, recent studies by the team's cardiovascular surgeon, Dr. Fred Grover, have established that this porous polyester mesh prosthetic material can be efficiently sutured, and that the sutures are stable under high stress conditions.

3. **Human Subjects:** No change

4. **Vertebrate Animals:** No change

5. **Publications:**

1. Wiesner TF, Levesque MJ, Rooz E, and Nerem RM. Epicardial coronary blood flow including the presence of stenoses and aorto-coronary bypasses. II: Experimental comparison and parametric investigations. ASME J Biomechl Engr. 110:2, 144-149, 1988.

2. Theret DP, Levesque MJ, Sato M, Nerem RM and Wheeler LT. The application of a homogeneous half-space model in the analysis of endothelial cell micropipette measurements. ASME J Biomech Engr 110:3, 190-199, 1988.

3. Levesque MJ, Sprague EA, Schwartz CJ and Nerem RM. The influence of shear stress on cultured vascular endothelial cells: The stress

response of an anchorage-dependent mammalian cell. Biotechnology Progress, 5:1-8, 1989.

4. Schwartz CJ, Sprague EA, Valente AJ, Kelley JL and Edwards, EH. Cellular mechanisms in the response of the arterial wall to injury and repair. Toxic Path. 17:66-71, 1989.

5. Schwartz CJ, Kelley JL, Nerem RM, Sprague EA, Rozez MM, Valente AJ, Edwards EH, Prasad ARS, Kerbacher JJ, and Logan SA. Pathophysiology of the atherogenic process. Am J Cardiol, 64:23G-30G, 1989.

6. Edwards EH, Sprague EA, Kelley JL, Kerbacher JJ, Schwartz CJ, and Elbein AD. Castanospermine inhibits the function of the low-density lipoprotein receptor. Biochemistry 28:7679-7687, 1989.

7. Samples DR, Sprague EA, Harper MJK and Herlihy JT. *In vitro* adsorption losses of arachidonic acid and calcium ionophore, A23187. Accepted, Am. J. Physiol., 1989.

8. Levesque MJ and Nerem RM. The study of rheological effects on vascular endothelial cells in culture. Biorheology 26:2, 345-357, 1989.

9. Nerem RM, Levesque MJ, Logan SA, Schwartz CJ and Sprague EA. Biologic responses of vascular endothelial cells to shear stress. Proceedings of the 8th International Symposium on Atherosclerosis, Rome, Italy, October 9-13, 1988. G Crepaldi, AM Gotto, E Manzato and G Baggio, eds. *Atherosclerosis VIII*. Elsevier Science Pub BV, pp 421-424, 1989.

10. Cayatte AJ, Schwartz CJ, Nerem RM, Rozek MM, and Sprague EA. Decreased adherence of platelets and monocytes to pre-endothelialized porous poloyester mesh exposed to prolonged elevated shear stress. In: *Cardiovascular Science and Technology: Basic & Applied: I.* J. Norman, ed. Oxymoron Press, Louisville, Kentucky, pp 57-59, 1989.

11. Sprague EA, Prasad ARS, Nerem RM, and Schwartz CJ. Elevated shear stress stimulated phosphoinositide-associated signal transduction pathways in cultured bovine aortic endothelial cells. In:

Cardiovascular Science and Technology: Basic & Applied: I. J. Norman, ed. Oxymoron Press, Louisville, Kentucky, pp. 71-73, 1989.

12. Nerem RM, Levesque MJ, Schwartz CJ, Acosta Y, and Sprague EA. Vascular endothelial cell proliferation the influence of physical environment. In: *Cardiovascular Science and Technolog: Basic & Applied: I.* J. Norman, ed. Oxymoron Press, Louisville, Kentucky, pp. 74-75, 1989.

13. Sprague E.A., Nerem, R.M., and Schwartz, C.J. Cellular recognition and transduction of fluid mechanical shear stress signals. In: Liepsch, D. ed. Proceedings of 2nd International Symposium on Biofluid Mechanics and Biorheology. In press, 1989.

14. Berliner JA, Valente AJ, Territo MC, Mahamad N, Parhami F, Gerrity RG, Schwartz CJ and Fogelman AM. Monocyte chemotactic factor produced by both stimulated and unstimulated endothelial cells is immunologically related to the factor produced by smooth muscle Cells. Submitted, J Clin Invest, 1989.

15. Cornhill JF, Sprague EA and Schwartz CJ. A morphometric approach to assess the response of endothelial ultrastructure to the multivalent ligand, cationized ferritin in areas of differing permeability to proteins. In preparation, 1990.

16. Kelley JL, Kerbacher JJ, Gilchrist EP, Rozek MM, Sprague EA and Schwartz CJ. Purification of the 'Scavenger' receptor for chemically modified lipoproteins from rabbit carrageenan granuloma macrophages. In Preparation, 1990.

17. Valente AJ, Rozek MM, Graves DT and Schwartz CJ. Identification of receptors for smooth muscle cell-derived chemotactic factor on peripheral blood monocytes. In preparation. 1990.

18. Cayatte AJ, Nerem RM, Schwartz CJ, and Sprague EA. Inhibition of platelet and monocyte recruitment to cultured endothelial cells preconditioned to elevated shear stress. In preparation, 1990.

19. Prasad ARS, Nerem RM, Schwartz CJ, and Sprague EA. Stimulation of phosphoinositide hydrolysis in cultured endothelial cells exposed to elevated shear stress. In preparation, 1990.

Abstracts:

1. Sprague EA, Edwards EH, Logan S, Schwartz CJ and Nerem RM. Enhanced LDL Receptor Expression in Cultured Arterial Endothelial Cells Exposed to Elevated Fluid-Imposed Wall Shear Stress. Symposium on Engineering Approaches to Atherosclerosis, 1988.

2. Schwartz CJ, Sprague EA, Nerem RM and Grover FL. Vascular Healing: Cell Biology and Rheologic Factors. NIH Devices and Technology Branch, Program:33, 1988.

3. Prasad ARS, Schwartz CJ. and Sprague EA. Phosphoinositide Metabolism and Low Density Lipoprotein Receptor-Mediated Endocytosis. Circulation 78:484a, 1988.

4. Kelley JL, Kerbacher JJ and Schwartz CJ. Purification to Homogeneity of the Acetyl-LDL "Scavenger" Receptor from Rabbit Carrageenan Granulomas. Circulation 78:13a, 1988.

5. Sprague EA, Prasad ARS and Schwartz CJ. Role of Signal Transduction in Low Density Lipoprotein (LDL) Receptor-Mediated Endocytosis. J Cell Biol, 107:810a, 1989.

6. Schwartz CJ. Pathophysiology of the Atherogenic Process. Presented at the Lipid Dynamics and Atherosclerosis Conference, 1989.

7. Sprague EA, and Prasad ARS. Modulation of LDL Receptor-Mediated Endocytosis by Agents Influencing PI Hydrolysis in Cultured Bovine Aortic Endothelial Cells. J Cell Biol, 109:139a, 1989.

8. Prasad ARS, Nerem RM, Schwartz CJ, and Sprague EA. Stimulation of Phosphoinositide Hydrolysis in Bovine Aortic Endothelial Cells Exposed to Elevated Shear Stress. J Cell Biol, 109:313a, 1989.

9. Berliner JA, Valente AJ, Territo MC, Mahamad N, Gerrity RG, Schwartz CJ and Fogelman AM. Monocyte Chemotactic Factor Produced by Endothelial Cells (EC) are Immunologically Related to the Factor Produced by Smooth Muscle Cells (SMC). Arteriosclerosis, 9:697a, 1989.

10. Kelley JL, Suenram CA, Rozek MM and Schwartz CJ. Granuloma Macrophages from WHHL Rabbits Accumulate Esterified Cholesterol and Become Foam Cells in the Absence of a Functional LDL Receptor. Arteriosclerosis, 9:744a, 1989.

11. Sprague EA, Cayatte AJ, Rozek MM, Nerem RM, and Schwartz CJ. Modulation of Platelet and Monocyte Adherence in Response to Elevated Shear Stress in Cultured Bovine Aortic Endothelial Cells. To be presented at the First World Congress of Biomechanics, UCSD, La Jolla, CA, August 30-September 4, 1990.

Book Chapters:

1. Gwyne JT and Schwartz CJ. (eds). A Symposium: Second International Conference on Hypercholesterolemia. Examining New Data on Probucol After a Decade of Use. Amer J Cardiol, 62:1B-81B, 1988.

2. Schwartz CJ. Perspectives on Coronary Artery Disease: Aetiology, Pathogenesis and Unresolved Problems. In: WJ Cliff and GI Schoefl, eds. *Coronaries and Cholesterol.* Chapman and Hall Medical, London, pp.1-25, 1989.

3. Schwartz CJ, Sprague EA, Valente AJ, Kelley JL, Edwards EH and Suenram CA. Inflammatory Components of the Human Atherosclerotic Plaque. In: S Glagov, WP Newman, III and SA Schaffer, eds. *Pathobiology of the Human Atherosclerotic Plaque.* Springer-Verlag New York, NY, pp. 107-120, 1989.